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14. ABSTRACT Our goal is to eliminate the tumor by vaccination and local ablation to render long-term immune protection without excessive autoimmune sequelae. Complimenting this regimen is systemic modulation of natural/induced Treg (iTreg) and intratumoral expression of immune augmenting cytokines. The two aims are to (1) Test the hypothesis that cryosurgery of cytokine enriched tumors amplifies Her-2 vaccine response, and (2) Test the hypothesis that disabling iTreg conversion enhances Her-2 immunity, not autoimmunity. In aim 1, we will measure immune response to tumor associated Her-2 by cryotherapy with and without DNA vaccination and the effect of intratumoral expression of cytokine. In aim 2, we will evaluate the degree of iTreg conversion in tumor bearing mice, measure Her-2 vaccine response in Her-2+TIEG1-/- mice which do not generate iTreg, and measure the induction of experimental autoimmune thyroiditis in iTreg deficient mice.					
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INTRODUCTION

Our goal is to eliminate the tumor by combining vaccination with local ablation to render long-term immune protection without excessive autoimmune sequelae. Complimenting this regimen is systemic modulation of regulatory T cells (Treg) and intratumoral expression of immune augmenting cytokines. We are testing two related hypotheses in the specific aims.

Aim 1 Test the hypothesis that cryosurgery of cytokine enriched tumors amplifies Her-2 vaccine response.

Aim 2 Test the hypothesis that disabling iTreg conversion enhances Her-2 immunity, not autoimmunity

BODY

Aim 1 Test the hypothesis that cryosurgery of cytokine enriched tumors amplifies Her-2 vaccine response.

To test the impact of cryoablation on tumor immunity, neu+ mammary tumor TUBO in BALB/c or BALB NeuT transgenic mice were cryoablated at the size of ~ 4x7 mm. In BALB/c mice approximately 10 µg/ml anti-neu antibodies were induced by cryoablation. Peritumoral injection with 100 µg CpG induced ~ 20 µg/ml antibodies. Combined treatment resulted in over 50 µg/ml of antibodies, showing enhanced immune response by TLR9 activation. Consistent with this finding, cryoablation-CpG combination therapy protected 7/8 mice from tumor re-challenge compared to 4/7 mice in cryoablation alone group. Therefore, significant tumor immunity was induced by cryoablation and further enhance by peritumoral CpG treatment. Rat neu expressed by TUBO tumor is, however, a foreign antigen in mice and may contribute to the strong immune reactivity.

It is important to test the impact of TUBO tumor cryoablation in BALB NeuT (NeuT) mice that express rat neu as a self antigen to mimic immune tolerance in humans and female mice develop spontaneous mammary tumors. NeuT females were electrovaccinated 12 days after s.c. TUBO inoculation with neu DNA. The implanted tumor was eliminated by cryoablation or surgical resection and mice were monitored for spontaneous tumor formation. Figure 2 shows minor elevation of anti-neu Ab in cryoablation group vs sham treated mice at 4 wks after tumor ablation (mouse age 110 days). Development of spontaneous tumor was, however, not altered. Surgical ablation after therapeutic vaccination, on the other hand, resulted in significant elevation of neu Ab and a modest, but significant delay in spontaneous tumor formation. Therefore, in immune tolerant host, surgical resection after therapeutic vaccination renders greater anti-tumor immunity than cryoablation.

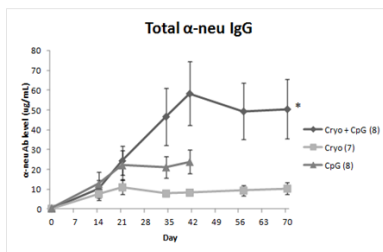


Figure 1 BALB/c mice inoculated s.c. with TUBO received cryoablation with or without peritumoral CpG. α-neu Ab in serum was measured by flow cytometry.

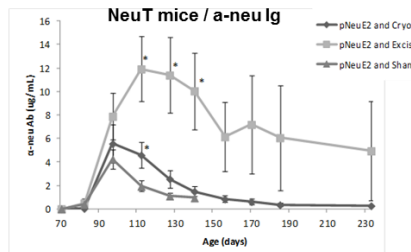


Figure 2 Tumor excision elevates rat neu specific Ab in tolerant NeuT mice. Serum was collected for α-neu Ab quantification via flow cytometry (n=8-9).

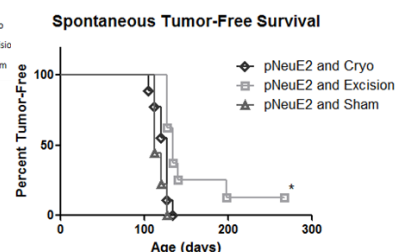


Figure 3 Development of spontaneous mammary tumor in NeuT female mice after indicated treatment.

Aim 2 Test the hypothesis that disabling iTreg conversion enhances Her-2 immunity, not autoimmunity

We tested immune response to Her-2 in C57BL/6 mice lacking the TGF-β-Inducible Early Gene 1 (TIEG1) to disable TGF-β mediated conversion of naïve CD4 + T to iTreg. After three time

electrovaccination with Her-2 DNA, Her-2-specific IFN- γ producing T cells in spleens of TIEG1 $^{-/-}$ mice was significantly higher than that in wild type mice, consistent with enhanced immune response in the absence of iTreg. Surprisingly, IgG response to Her-2 DNA vaccine was significantly lower in TIEG1 $^{-/-}$ mice starting from the first vaccination and continuing throughout the experiment. Therefore, TIEG1 deficiency results in enhanced T cell response, but reduced antibody production.

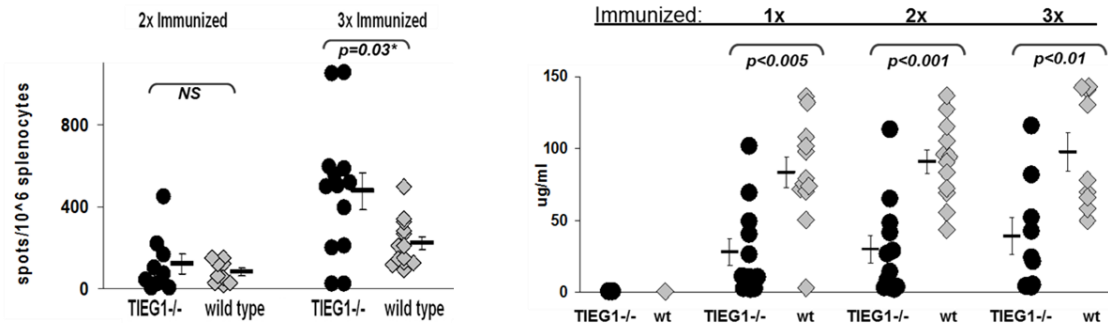


Figure Stronger IFN- γ T cell response and lower antibody production in TIEG1 $^{-/-}$ mice vaccinated with Her-2 DNA. TIEG1 $^{-/-}$ and wild type mice were electro-vaccinated with Her-2 DNA at 2 week intervals. Her-2-specific antibody and T cell responses were measured by flow cytometry and ELISPOT respectively. A. Her-2-specific IFN- γ -producing T cells in ELISPOT (*p value shown for Kruskal-Wallis test). B. anti-Her-2 IgG antibody levels measured by flow cytometry.

KEY RESEARCH ACCOMPLISHMENTS

1. Characterized immune response to tumor associated neu by cryoablation, peritumoral CPG treatment and surgical resection in wild type vs immune tolerant mice to show boosting of vaccine response only by surgical resection in immune tolerant hosts.
2. Demonstrated enhanced T cell response in TIEG1 $^{-/-}$ mice that are impaired in iTreg. These mice, however, are defective in antibody production to indicate additional alteration by TIEG1 inactivation.

REPORTABLE OUTCOMES

1. Veenstra J., Gibson H., Littrup P., Wei W. 2012. Modulation of vaccine induced tumor immunity with cryoablation in wild type and immune tolerant hosts. Translational Research Cancer Center Consortium. Seven Springs, PA.

CONCLUSIONS

In BALB/c mice, cryoablation and peritumoral CpG treatment of neu+ TUBO tumor showed synergistic induction of anti-neu antibody and protection against tumor re-challenge. In neu tolerant mice, cryoablation showed little enhancement of vaccine induced anti-neu immunity. Surgical resection of the tumor after vaccination induced greater antibody response to delay spontaneous tumorigenesis. Taken together, surgical resection is advantageous over cryoablation in boosting vaccine response in tolerant hosts.

TIEG1 deficiency results in impaired iTreg conversion. Enhanced T cell response to Her-2 DNA vaccination may be attributed to the absence of iTreg. The surprising finding of profound reduction in antibody production warrants further investigation.